

Figure 1 (SEQ ID NO:724)

85P1B3 SSH sequence and GenBank homology to OIP5

GATCAGAGGACACATGGGACTCTGCATCTTAATTCCTAAATTTACAGTCAAAGACATTTTCAG AGATAAGTATTATGAATTCAATAAGAATCTAAAGTAAGTTCTTAAGGCAAATAGCTATAAAA GAGAAGAATCCTTAGTCTCTCATCTTCTAAAAACAGCTTCACAAATAATTTGGAAAAATCAGCC TAAAGGTAAATAGAAACTGCATTTCCCCTCCATTCTTGAAGCCAATCTTTTTCAAGAAATGAC TAAGCAGCACCTGTTGTTGAAGACAGCAATAAAGCCTGAACCTGACACTCAAGCTTTGGTACA GGATC

gb|AF025441.1|AF025441 Homo sapiens Opa-interacting protein... 632 e-179 gb|AF158642.1|AF158642 Homo sapiens metalloproteinase-disin... 42 0.12 gb|AC005075.2|AC005075 Homo sapiens clone RG219E16, complet... 42 0.12 emb|AL096773.6|HS1000E10 Human DNA sequence from clone 1000... 40 0.48

>gb|AF025441.1|AF025441 Homo sapiens Opa-interacting protein OIP5 mRNA, partial cds Length = 1197

Score = 632 bits (319), Expect = e-179 Identities = 319/319 (100%) Strand = Plus / Minus

gatcagaggacacatgggactctgcatcttaattcctaaatttacagtcaaagacatttt 60 Query: 1 Sbjct: 1013 gatcagaggacacatgggactctgcatcttaattcctaaatttacagtcaaagacatttt 954 Query: 61 Sbjct: 953 taaaagagaagaatccttagtctctcatcttctaaaaacagcttcacaaataatttggaa 180 Query: 121 taaaagagaagaatccttagtctctcatcttctaaaaacagcttcacaaataatttggaa 834 Sbjct: 893 aatcagcctaaaggtaaatagaaactgcatttcccctccattcttgaagccaatcttttt 240 aatcagcctaaaggtaaatagaaactgcatttcccctccattcttgaagccaatctttt 774 Sbjct: 833 caagaaatgactaagcagcacctgttgttgaagacagcaataaagcctgaacctgacact 300 Query: 241 caagaaatgactaagcagcactgttgttgaagacagcaataaagcctgaacctgacact 714 Sbjct: 773 Query: 301 caagetttggtacaggate 319 (SEQ ID NO:725) 4111111111111111111 caagetttggtacaggate 695 (SEQ ID NO:726) Sbjct: 713

Sheet 2 of 22

Figure 2

↓) ID N	9			18			27			36			45			54
GGC	TGC		AAG	ATG		GCT	CAG		CTG	CGG	CAT	CGC	TCA		TGT	GCA	
	(SEQ	ID NO	 :728)→	м	A	A	Q	P	L	R	H	R	s	R	С	A	т
		63			72			81			90			.99		•	108
CCG	ccc	CGG	GGG	GAC	TTT	TGT	GGT	GGC	ACT	GAG	AGG	GCG	ATT	GAC	CAA	GCT	TC
P	P	R	G	D	F	С	G	G	T	E	R	A	I	Đ	Q	A	S
		117			126			135			144			153			16:
TTT	ACG	ACC	TCC	ATG	GAG	TGG	GAT	ACG	CAG	GTG	GTG	AAG	GGG	TCC	TCG	CCG	CT
F	T	T	s	M	E	W	D	T	Q.	V	V	K	G	.s	s	P .	L
		171			180			189			198			207			21
GGC	CCC	GCA	GGG	CTG	GGG	GCT	GAG	GAG	CCA	GCC	GCC	GGC	CCG	CAG	CTG	CCG	TC
G	P	A	G	L	G	A	E	E	P.	A	A	G	P	Q	L	P	s
		225		•	234			243			252			261			270
TGG	CTG	CAG	CCT	GAG	AGG	TGC	GCT	GTG	TTC	CAG	TGC	GCA	CAG	TGT	CAC	GCA	GTY
W	T	Q	P	E	R	С	A	v	F	Q	C	A	Q	C	н	A	V
		279			288			297	~.~		306			315			32
CTC	GCC	GAC	TCG	GTG	CAC	CTC	GCC	TGG	GAC	CIG	TCG	CGG	TCC	CTC	GGG	GCC	GT
L	A	D	s	V	н	L	A	W	D	L	s	R	S	L	G	A	V
		333			342			351		5 000	360			369			37
GTC	TTC	TCC	AGA	GTT	ACA	AAT	AAC	GIC	GTT,	11G	GAA	GCG		TIC	CTA	GTT	GG(
V	F	S	R	V	T	N	N	V	V	L	E	A	P	F	L	v	G
እ ጥጥ	CAA	387	m~ a	CTYC	396	ccc	λст	405	ሞልሮ	220	414 CTT	מיחים	en-en-cr	423	CCT	m/m	43
Ι	E	G	s	L	K	G	S	T	Y	N	L	L	F	Ċ	G	s	С
ccc	ልጥጥ	441		CCT	450	СУТ	CTG	459	ጥጥ		468 CAT	ርርጥ	CCC	477		GCC	48
			·	<u>-</u> -													
G	Ι	P	.₩	G	F	н	L	¥			H	A	A	, L	A	A	L
AGA	GGT	495		TYGC	504 CTT		AGT	513 GAC		ATG	522 GTG		тат	531 CTC		ÁΑΑ	54 AC
R	G	H	F	С	. L	.5	S	D	K	M	V	С	Y	L	L	K	T
AAA	GCC	549 ATA		ААТ	558 GCA	TCA	GAG	567 ATG		ል ሞዮ	576 CAA	AAT	GTT.	585 CCT	ርጥል	TCA	59 GA
														·			
K	A	1	V	N	A	s		M		ī	Q	N	V	ħ	L	S	E
AAG	አጥጥ	603 GCA		CTC	612 AAA		AAG	621 ATA		СТА	630 ACG	CAC	ልልጥ		Т ТА		64 TC
			E				K				T						S

Sheet 3 of 22

		657			666			675			684			693			702
CTA	ATG	AAG	ATT	CTG	AGT	GAA	GTG	ACT	CCT	GAC	CAG	TCC	AAG	CCA	GAA	AAC	TGA
1.	M	V	1	L	s	£	V	T	P	D	Q	S	K	P	E	N	*
		711			720			720			720			242			
TCC	TGT	ACC	AAA	GCT	TGA	GTG	TΥ	CCT	TYC A	ccc	128	a mm	CCM	747	mma		756
		765			774			783			792			801			810
AGG	TGC	TGC	TTA	GTC	ATT	TCT	TGA	AÁA	AGA	TTG	GCT	TCA	AGA	ATG	GAG	GGG	AAA
		819			828			837			846			855			864
TGC	AGT	TTC	TAT	TTA	CCT	TTA	GGC	TGA	TTT	TCC	AAA	TTA	TTT	GTG	AAG	CTG	TTT
ע יווייזי	CAA	873	CNC	303	882 Cm3	300	3 000	891	ama	~~~	900			909			918
		GAI	GAG	AGA	CTA	AGG	ATT							TTG			
		927			936			945									
СТТ	ACT	TTA	GAT	TCT	TAT	TGA	АТТ	CAT	ААТ	АСТ	TAT	כיזיכי	TCA	202	mCm.	Curr	972 TV:3
		981			990			999			1008			1017		1	1026
CTG,	TĄA.	ATT	TAG	GAA	TTA	AGA	TGC	AGA	GTC	CCA	TGT	GTC	CTC	TGA	TCT	AAA	GTT
-,-																	
	1	1035		:	1044		:	1053			1062			1071		1	L080
GCA	TGG	TTG	GTC	TGA	AAA	TAG	AGT	TGG	GCT	TAA							
								1107									
тап	GGA ,	עטט	GTT	CTTT.	L098 ATG	አአጥ	እርጥ	7 7 7 T	303	max.	1116	mmm		1125]	1134
							ACI	AAI	ACA	1CA	CTT	TŢŢ	AAC	TIC	TGT	AAA	ATA
	1	1143		-	1152			1161			1170			1170			100
CAG	ATC	ATA	ATA	TTC	TAT	AGG	TAA	TGT	TTA	ATA	AAT	TGC	CTG	TAAT	አ ልጉ	444	777
	1	1197		:	L206		:	1215		1	L224		1	L233		1	242
AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA	AAA
AAA	AAA	AAA	AAA	AAA	AAA	AA 3	, ·										

Sheet 4 of 22

Figure 3

85P1B3/OIP5 protein sequence. (SEQ ID NO:729)

- 1 MAAQPLRHRS RCATPPRGDF CGGTERAIDQ ASFTTSMEWD TQVVKGSSPL GPAGLGAEEP 61 AAGPQLPSWL QPERCAVFQC AQCHAVLADS VHLAWDLSRS LGAVVFSRVT NNVVLEAPFL 121 VGIEGSLKGS TYNLLFCGSC GIPVGFHLYS THAALAALRG HFCLSSDKMV CYLLKTKAIV 181 NASEMDIQNV PLSEKIAELK EKIVLTHNRL KSLMKILSEV TPDQSKPEN*

Sheet 5 of 22

Figure 4

Alignment of 85P1B3 with OIP5.

>gi|2815610|gb|AAC39561.1| (AF025441) Opa-interacting protein OIP5 [Homo sapiens] Length = 231

Score = 462 bits (1189), Expect = e-130Identities = 229/229 (100%), Positives = 229/229 (100%)

85P1B3: 1 MAAQPLRHRSRCATPPRGDFCGGTERAIDQASFTTSMEWDTQVVKGSSPLGPAGLGAEEP 60 MAAQPLRHRSRCATPPRGDFCGGTERAIDQASFTTSMEWDTQVVKGSSPLGPAGLGAEEP 62 MAAQPLRHRSRCATPPRGDFCGGTERAIDQASFTTSMEWDTQVVKGSSPLGPAGLGAEEP 62

85P1B3: 61 AAGPQLPSWLQPERCAVFQCAQCHAVLADSVHLAWDLSRSLGAVVFSRVTNNVVLEAPFL 120
AAGPQLPSWLQPERCAVFQCAQCHAVLADSVHLAWDLSRSLGAVVFSRVTNNVVLEAPFL
0IP5: 63 AAGPQLPSWLQPERCAVFQCAQCHAVLADSVHLAWDLSRSLGAVVFSRVTNNVVLEAPFL 122

85P1B3:121 VGIEGSLKGSTYNLLFCGSCGIPVGFHLYSTHAALAALRGHFCLSSDKMVCYLLKTKAIV 180 VGIEGSLKGSTYNLLFCGSCGIPVGFHLYSTHAALAALRGHFCLSSDKMVCYLLKTKAIV

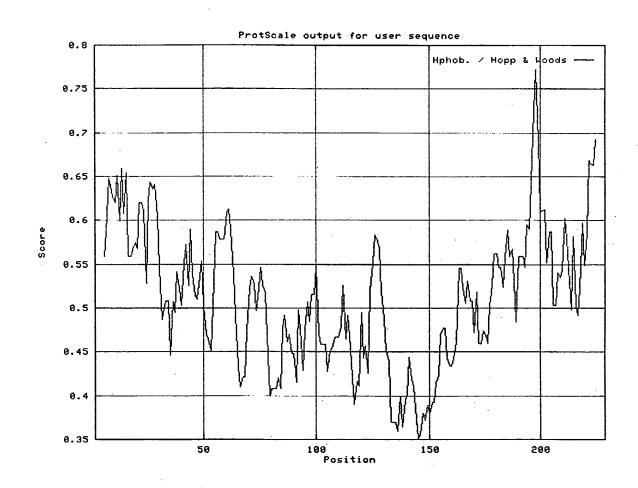
OIP5: 123 VGIEGSLKGSTYNLLFCGSCGIPVGFHLYSTHAALAALRGHFCLSSDKMVCYLLKTKAIV 182

85P1B3:181 NASEMDIQNVPLSEKIAELKEKIVLTHNRLKSLMKILSEVTPDQSKPEN 229 (SEQ ID NO:730)
NASEMDIQNVPLSEKIAELKEKIVLTHNRLKSLMKILSEVTPDQSKPEN

01P5: 183 NASEMDIQNVPLSEKIAELKEKIVLTHNRLKSLMKILSEVTPDQSKPEN 231 (SEQ ID NO:731)

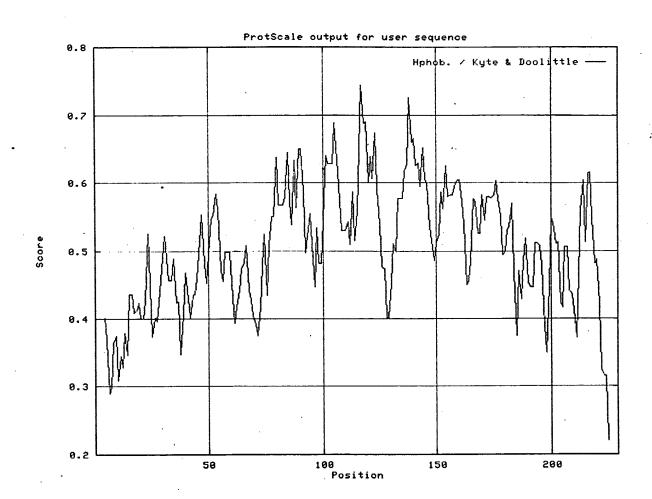
Sheet 6 of 22

Figure 5: 85P1B3 Hydrophilicity profile (Hopp T.P., Woods K.R., 1981. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828)



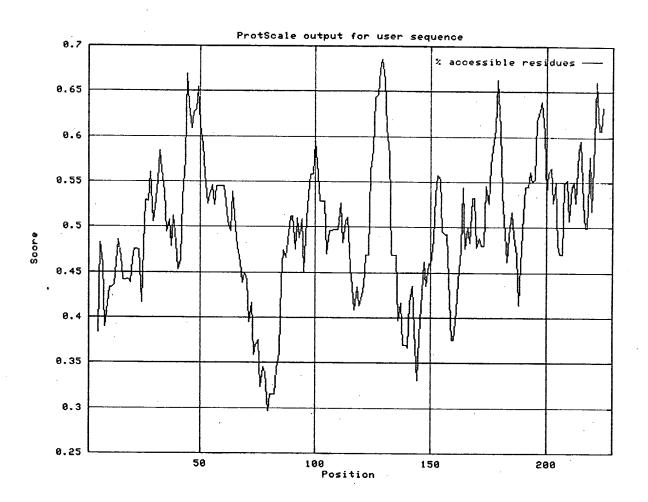
Sheet 7 of 22

Figure 6: 85P1B3 Hydropathicity Profile (Kyte J., Doolittle R.F., 1982. J. Mol. Biol. 157:105-132)



Sheet 8 of 22

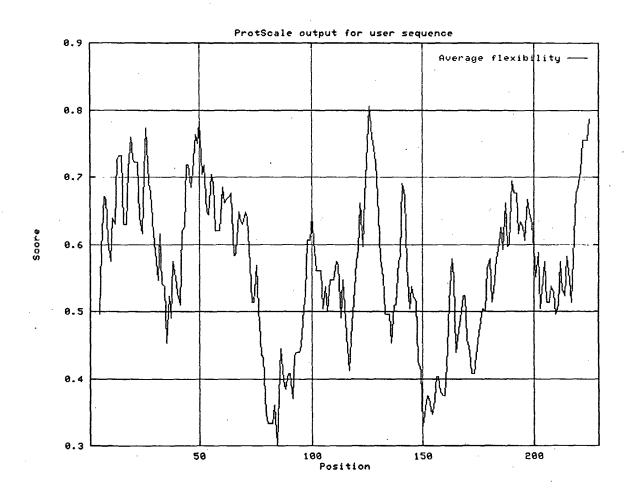
Figure 7: 85P1B3 % Accessible Residues Profile (Janin J., 1979. Nature 277:491-492)



Sheet 9 of 22

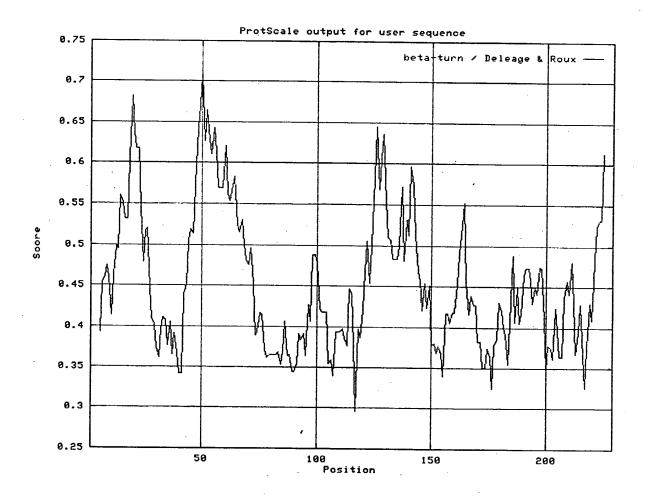
Figure 8: 85P1B3 Average Flexibility Profile

(Bhaskaran R., Ponnuswamy P.K., 1988. Int. J. Pept. Protein Res. 32:242-255)



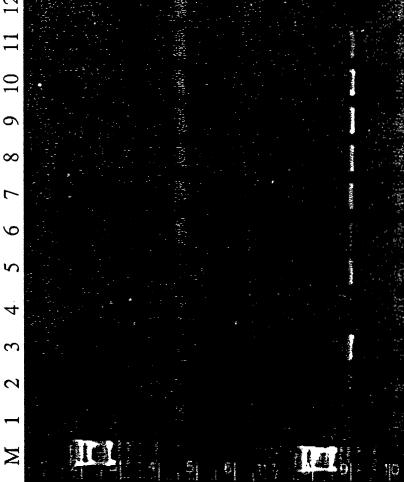
Sheet 10 of 22

Figure 9: 85P1B3 Beta-turn Profile (Deleage, G., Roux B. 1987. Protein Engineering 1:289-294)



Sheet 11 of 22

26X



4) Prostate Cancer Pool

3) Xenograft Pool

2) Vital Pool 2

1) Vital Pool

Bladder Cancer Pool

Kidney Cancer Pool

Colon Cancer Pool

Lung Cancer Pool

Metastasis Pool

Ovary Cancer Pool

Breast Cancer Pool

H20

Figure 10 RT-PCR analysis of 85P1B3 expression.

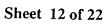
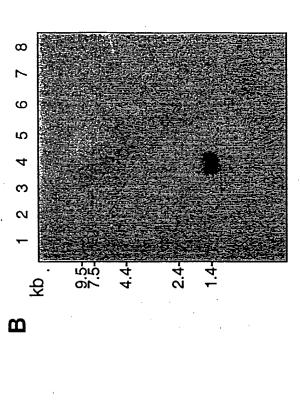
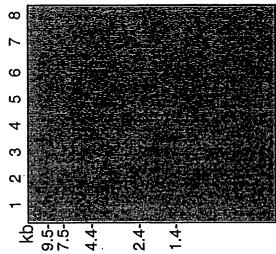


Figure 11 Expression of 85P1B3 in Normal Human Tissues







Heart
 Brain
 Placenta
 Lung
 Liver
 Skeletal Muscle
 Kidney
 Pancreas

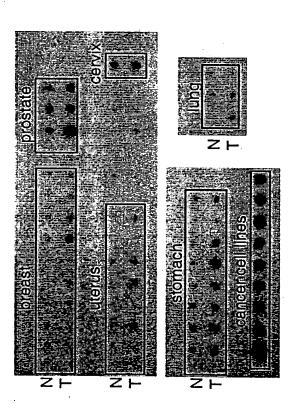
4

Sheet 13 of 22

50. Caco-2 51. LoVo 52. T84 53. Colo205 54. KCL 22 49. SK-COovarian 41 42 43 42. OV-1063 46. Bx PC-3 18. Capan-1 44. SW626 45. PANC-47. HPAC 43. PA-1 37 38 39 40 testicular 16 36. MDA-MB-435s 37. NTERRA-2 5 36 38. NCCIT 39. TERA-1 40. TERA-2 34. DU4475 33. C. AMA-1 35. MCF-7 breast 33 34 35 4 bladder 52 53 54 <u>ლ</u> 언 28. NCI-H146 29. 769-P 30. A498 31. CAKI-1 32. SW839 - 32 27. NCI-H82 colon 50 51 25. CALU-1 26. A427 29 30 31 9 kidney 49 45 46 47 48 σ pancreatic 28 19. PFSK-1 20. T98G 21. SK-ES-1 22. HOS 23. U2-OS 24. RD-ES prostate 27 lung ဖ 26 Ŋ 25 4 12. SCaBER 13. UM-UC-3 24 4. TCCSUP 즁 15. J82 16. 5637 17. RD-ES 18. 293T 11. HT1197 က bone 21 22 23 Q kb 1.4-19 20 brain 4. LAPC-9 AD 5. LAPC-9 AI 6. LNCaP 7. PC-3 8. DU145 9. TsuPr1 10. LAPC-4 CL 3. LAPC-4 AI 즁

Figure 12 Expression of 85P1B3 in Human Cancer Cell lines

Figure 13 Expression of 85P1B3 in Patient Cancer Specimens and Cancer **Cell Lines**



Cancer cell lines are: (from left to right)

HeLa (cervical carcinoma)
Daudi (Burkitt's lymphoma)
K562 (CML)
HL-60 (PML)
G361 (melanoma)
A549 (lung carcinoma)
MOLT-4 (lymphoblastic leuk.)

SW480 (colorectal carcinoma)

Raji (Burkitt's lymphoma)

T = tumor RNA N = normal adjacent tissue RNA

4 2

겅 ರ

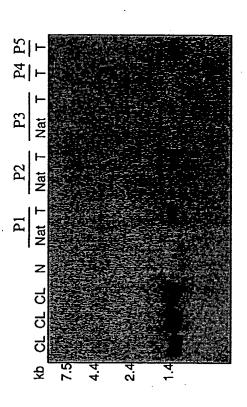
ರ

<u>용</u>

P1 – Stage III, T2N1MX P2 – Stage III, T3N1MX

Cell Lines (CL) - Colo 205, LoVo, T84, Caco-2 N = Normal Colon Nat = Normal Adjacent tissue T = Tumor

Figure 15 Expression of 85P1B3 in Bladder Cancer Patient Specimens



P1 - Transitional, grade 2 P2 - Transitional, grade 3/2

P3 - Transitional

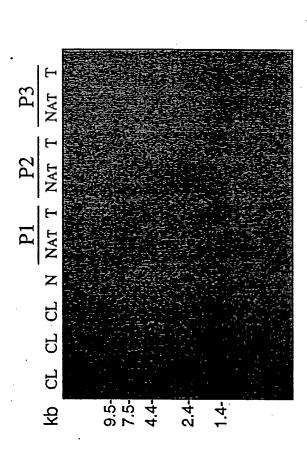
P4 - Polypoid cystitis P5 - Papillary, grade 3

CI = cell lines listed in order: UM-UC-3, J82, SCABER
P = Patient
N = Normal Bladder

Nat = Normal adjacent tumor T = Tumor

Sheet 17 of 22

Figure 16 Expression of 85P1B3 in Lung Cancer Patient Specimens



= cell lines (listed in order): A427, NCI-H82, NCI-H146

ರ

N = Normal Lung NAT = Normal adjacent tissue T = Tumor

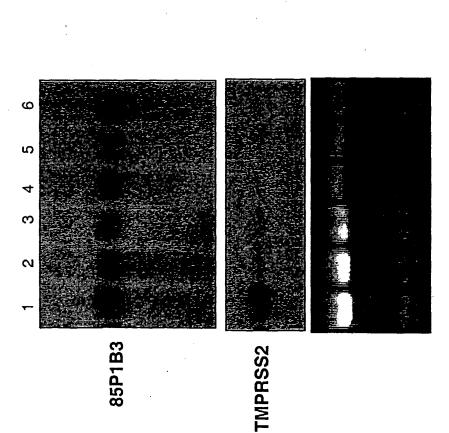
= Patient

Q 2

P1 - Squamous, stage IB P2 - Squamous, stage IIB P3 - Squamous, stage IIIA

Sheet 18 of 22

Figure 17 Expression of 85P1B3 in Prostate Cancer Xenografts Following Castration



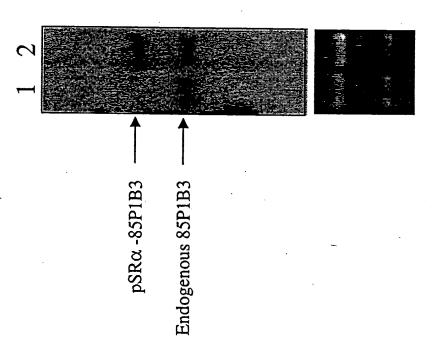
-APC-9AD Day 15 – Mouse #4 -APC-9AD Day 15 – Mouse #5

-APC-9AD Day 0 - Mouse #1 -APC-9AD Day 7 - Mouse #2 -APC-9AD Day 7 - Mouse #3 APC-9AD Day 21 - Mouse #6

Figure 18 Expression of 85P1B3 in PC3 Cells Following Retroviral-

Mediated Gene Delivery





Sheet 20 of 22

85P1B3 SSH sequence (701-1019) 1 19 101 Splice variant 1 (496 bp) **85P1B3** (1262 bp)

Figure 19.

